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Analytical method validation for the determination of assay of levetiracetam in levetiracetam injection formulation by HPLC

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ABSTRACT

To develop and validate a effective stability indicating HPLC procedure to assay levetiracetam in pure and applied on injection formulations containing levetiracetam.For chromatographic separation and analysis, the mobile phase consisted of potassium phosphate buffer (pH 5.5) and acetonitrile (45:55, v/v). The separation was achieved on an YMC-pack AQ (3µm, 150 × 4.6 mm) column in isocratic mode. The method was validated following the recommendations of ICH. The method for levetiracetam assay has been shown to be precise, accurate, specific, rugged and robust. The procedure was also proved as stability indicating, as the major degradation products and excipients were not interfering with levetiracetam assay. An effective stability indicating HPLC method has been developed and validated for the determination of levetiracetam in pure and applied successfully on injection formulations containing levetiracetam.

KEY WORDS: Levetiracetam, Injection formulation, Validation, Stability indicating, Chromatography, ICH Guidelines

INTRODUCTION

Levetiracetam is an antiepileptic and anticonvulsant agent belonging to pyrrolidine class of chemical molecules. Chemically as per IUPAC, levetiracetam is termed as (*S*)-2-(2-Oxopyrrolidin-1-yl) butanamide.[1,2]Levetiracetam is suggested in the management of partial onset seizures in epileptic patients aged one month and older as an adjunctive treatment. Levetiracetam is also indicated as an adjunctive treatment in the management of myoclonic seizures in patients aged 12 years and older. Furthermore it is recommended in treating primary generalized tonic clonic seizures in generalized idiopathic epilepsy patients aged 6 years and older.[3-5]The specific process by which levetiracetam show its antiepileptic effects are unclear. Few studies suggested that levetiracetam induces pre-synaptic SV2A (synaptic vesicle protein found throughout the central nervous system), leading to the inhibition of release of neurotransmitter. Thisactivity does not have an effect on normal neurotransmission.[6-8] Levetiracetam is available as extended release tablets, immediate release tablets, oral solution and injectable dosage form.[9]

Few methods are proposed to quantify levetiracetam in tablet dosage form. They are colorimetry,[10,11] UV spectrophotometry[11, 12] and HPLC.[13-16] Several methodologies for quantifying levetiracetam concentration in human serum or plasma have been developed. They are HPLC,[17-19] UPLC,[20] LC-MS,[21-24] UPLC-MS[25] and GC-MS.[26]Few analytical procedures for determining levetiracetam concentration in saliva was developed using LC-MS,[24] UPLC-MS[25] and GC-MS.[26]To the best of our information through online survey, there is no documented method to evaluate levetiracetam in injectable dosage forms utilizing stability indicating RP-HPLC. Hence, this investigation is aimed at developing and validating a stability indicating RP-HPLC method to assess levetiracetam content in injectable dosage forms.

MATERIALS AND METHODS

Equipments

- Shimadzu High performance liquid chromatography(Model no. LC201OCHT)

system equipped with auto sampler and photodiode array detector (PDA).
- Sartorius analytical balance (Model no: CP225D)
- Photo stability chamber (Model no. GDFUVMOI & GDFPHSOI)
- Hot air oven (Model no. GDFHA002 & GDFHA003)
- Metrohm pH meter (Model no: pH780)
- YMC-pack AQ (3µm, 150 × 4.6mm)

Reference Drugs and Injectable Dosage Forms

- Levetiracetam reference drug (Batch no: 08-0580912)
- Levetiracetam in sodium chloride injection dosage form (Batch no. LVC-OI-037, label claim 5.0 mg/ml; Batch no. LVC-OI-039, label claim 10 mg/ml; and Batch no. LVC-OI-04, label claim 15 mg/ml)

Solvents and Chemicals

- Analytical grade monobasic potassium phosphate, Rankem avantor chemicals.
- Analytical grade potassium hydroxide, Merck specialties ltd.
- HPLC grade acetonitrile, Fischer scientific ltd.

HPLC Conditions

- Mobile phase: potassium phosphate buffer (pH 5.5) and acetonitrile (45:55, v/v)
- Flow rate: 0.9 ml/min
- Temperature: 20 ºC
- Elution: isocratic mode
- wavelength for quantification: 205 nm

Preparation of Solutions

Calibration Solutions

Levetiracetam stock solution (1.0380 mg/ml) was made by transferring accurately weighed 25.95 mg levetiracetam reference drug to a 25 ml flask, dissolved and finally diluted to with diluent to volume. For linearity, five concentration levels over a range of 50% level (0.519 mg/ml) to 150% level (0.1557 mg/ml) of the test concentration of 0.1 mg/ml of levetiracetam were prepared from stock with apt dilution with diluent.

Accuracy Study Solutions

Excipient stock solution was prepared by dissolving 410.17 mg sodium chloride, 82.25 mg sodium acetate trihydrate and 2.76 mg of glacial acetic acid in 40 ml of water and mixed thoroughly in a 50 ml flask. The solution was buffered at pH 5.5 with glacial acetic acid and diluted to mark with water. Excipient stock solution (placebo) was spiked with levetiracetam reference drug at 3 concentration levels (50% - 0.0507 mg/ml levetiracetam, 100% - 0.1014 mg/ml levetiracetam and 150% - 0.1521 mg/ml levetiracetam).

Solutions for Precision, System Suitability and Robustness

Levetiracetam stock solution (1.0380 mg/ml) was made by transferring accurately weighed 25.95 mg levetiracetam reference drug to a 25 ml flask, dissolved and finally diluted to with diluent to volume. 100% concentration level (0.1038 mg/ml) solutions were prepared from stock with apt dilution with diluent.

Injection Sample Solution

Exactly transferred 1.0 ml of injection dosage form (label claim - 5mg/ml) to a 50ml flaskand diluted to mark with diluent. Concentration of the resultant solution is 0.1 mg/ml. For injection dosage forms with label claim 10 mg/ml and 15 mg/ml, the injection sample solution (0.1mg/ml) for analysis was prepared by appropriate dilution of the injection dosage form with diluent.

Forced Degradation Samples

Dry Heat Degradation

Five ml of levetiracetam injection sample was exposed to heat stress by placing the sample in an oven set at 80 ºC for 7 days. For analysis, exactly transferred 1.0 ml of degraded sample to a 50 ml flask and diluted to mark with diluent.

UV Degradation

Five 5 ml of levetiracetam injection sample was transferred to a stoppered test tube and exposed to 1.2 million Lux hours illumination of cool fluorescent light and an UV energy of 200 watt hours/m^2 simultaneously in a photo stability chamber which was set at 25ºC. For analysis, exactly transferred 1.0 ml of degraded

sample to a 50 ml flask and diluted to mark with diluent.

Acid Degradation

41.03 mg of sodium acetate, 205.45 mg of sodium chloride and 1.41 mg of glacial acetic acid were dissolved in 10 ml of water. The solution pH was adjusted to 5.5 with 1M acetic acid. 125.0 mg of levetiracetam reference drug was dissolved in the above solution. Again checked and adjusted the solution pH to 5.5 with 1M acetic acid. To this mixture solution, 10 ml of 0.2 N hydrochloric acid was added and stored at 50 ^{o}C for 3 hr. After the specified period of degradation, readjusted the pH to 5.5 with 1N NaOH. The solution was quantitatively made up to 25 ml with water. For analysis, exactly transferred 1.0 ml of degraded sample to a 50 ml flask and diluted to mark with diluent.

Base Degradation

41.70 mg of sodium acetate, 205.84 mg of sodium chloride and 1.47 mg of glacial acetic acid were dissolved in 10 ml of water. The solution pH was adjusted to 5.5 with 1M acetic acid. 125.25 mg of levetiracetam reference drug was dissolved in the above solution. Again checked and adjusted the solution pH to 5.5 with 1M acetic acid. To this mixture solution, 10 ml of 0.2 N sodium hydroxide was added and stored at room temperature for 2 hr. After the specified period of degradation, readjusted the pH to 5.5 with 1N HCl. The solution was quantitatively made up to 25 ml with water. For analysis, exactly transferred 1.0 ml of degraded sample to a 50 ml flask and diluted to mark with diluent.

Peroxide Degradation

41.07 mg of sodium acetate, 205.25 mg of sodium chloride and 1.41 mg of glacial acetic acid were dissolved in 10 ml of water. The solution pH was adjusted to 5.5 with 1M acetic acid. 124.84 mg of levetiracetam reference drug was dissolved in the above solution. Again checked and adjusted the solution pH to 5.5 with 1M acetic acid. To this mixture solution, 10 ml of 6% peroxide was added and stored at 100 ^{o}C for 30 min. After 30 min, the solution was quantitatively made up to 25 ml with water. For analysis, exactly transferred 1.0 ml of degraded sample to a 50 ml flask and diluted to mark with diluent.

Procedures

Calibration Curve for Levetiracetam

The calibration solutions with levetiracetam concentrations 0.0519 mg/ml, 0.0779 mg/ml, 0.1038 mg/ml, 0.1298 mg/ml and 0.1557 mg/ml were prepared. These solutions were evaluated using the suggested method. A plot of levetiracetam concentration *vs* levetiracetam response (area) was built. A linear regression analysis was performed on the data obtained (concentration and peak response) to get regression equation and regression coefficient.

Assay of Levetiracetam in Injection Dosage Form

The sample prepared in section "Injection sample solution" was analyzed using the suggested method. The levetiracetam response (area) was determined. The content of levetiracetam was identified using calibration curve or regression equation.

Degradation Study

The forced degradation samples of injection dosage form and excipient placebo spiked levetiracetam reference drug were used to display the suggested method's stability indicating characteristics and specificity. The control (undegraded injection sample solution), dry heat and light exposed injection samples prepared were used in this study. Excipient placebo solutions spiked with levetiracetam exposed to acid, base and peroxide stress conditions were also used in this study. The forced degraded samples were evaluated using the suggested method.

Photodiode array detector was used to evaluate the peak homogeneity/peak purity and spectral match. Peak homogeneity was ascertained by making comparisons of UV spectra acquired atdifferent points within the peak of levetiracetam in degraded samples. Spectral match was ascertained by making comparisons of UV spectrum acquired from peak apex of levetiracetam in degraded sample with peak apex of levetiracetam undegraded standard solution.

RESULTS AND DISCUSSION

Validation of the Suggested Method

Validation parameters (system suitability, linearity, sensitivity, precision, accuracy, robustness, ruggedness, specificity and selectivity) were validated following criteria of International Conference on Harmonization.[27]

Before each analysis, this was done to make sure that the system is best suited for levetiracetam analysis in dosage forms of injection. System suitability parameters like repeatability and number of theoretical plates were assessed by injection offive replicates of levetiracetam standard solutions (0.1 mg/ml). The results confirmed that the system met the criteria for suitability [Table 1].

System Suitability

Table 1: Levetiracetam system suitability details

Inj. No.	Peak area	Statistical assessment	Criteria for acceptance
Inj. 1	2228299	**Average:**	RSD percent for five replicates of standard solution - ≤ 2.0%
Inj. 2	2230465	2229781	
Inj. 3	2230925	**RSD percent:**	
Inj. 4	2230091	0.05	
Inj. 5	2229126		
Plate count		58854	Should be not less than 20000

Inj. – injection; No. – number; RSD – relative standard deviation

Linearity and Range

Standard linearity was conducted to evaluate to see if a single point calibration could provide adequate accuracy over the method's intended operating range. For standard linearity, five levels of concentration were assessed over a range of 50% level (0.0519 mg/ml) to 150% level (0.1557 mg/ml) of the test concentration (0.1 mg/ml of levetiracetam). Linearity results for standard levetiracetam are shown in Table 2. The results confirmed that the procedure met the criteria (regression coefficient was >0.999) for linearity in the range of 0.0519 mg/ml to 0.1557 mg/ml.

Table 2: Levetiracetam linearity details

Level (with relating to test concentration – 0.1 mg/ml)	Concentration (mg/ml)	Area response
50	0.0519	1173413
75	0.0779	1748966
100	01038	2294224
125	0.1298	2846245
150	0.1557	3362764
Regression equation	y = 21123169.41219 c + 94730	
Regression coefficient	0.9996	

y = area response; c = concentration (mg/ml)

Precision

System Precision

Injected the standard solution (0.1 mg/ml levetiracetam) 6 times and determined the percent RSD of area and retention time of levetiracetam peak. The findings are shown in Table 3. The results confirmed that the procedure met the criteria (percent RSD was <2.0%) for system precision.

Table 3: Levetiracetam system precision details

Inj. No.	Area response	Statistical assessment	Retention time	Statistical assessment
Inj. 1	2231098	**Average:** 2230799	10.338	**Average:** 10.318
Inj. 2	2229853		10.335	
Inj. 3	2230206		10.329	
Inj. 4	2230915	**RSD percent:** 0.04	10.314	**RSD percent:** 0.20
Inj. 5	2230599		10.305	
Inj. 6	2232123		10.287	

Inj. – injection; No. – number; RSD – relative standard deviation

Method Precision

Six injection formulation sample solutions were prepared at concentration level of 100% (0.1 mg/ml). For every sample, the assay of levetiracetam according to the developed method was determined. The percent RSD for assay results were assessed. The findings are shown in Table 4. The results confirmed that the procedure met the criteria (percent RSD was <2.0%) for method precision.

Table 4: Levetiracetam system precision details

Injection formulation with label claim 5 mg/ml			Injection formulation with label claim 15 mg/ml		
Concentration (mg/ml)	**Assay (%)**	**Statistical assessment**	**Concentration (mg/ml)**	**Assay (%)**	**Statistical assessment**
0.1	100.0	**Average:** 100.1	0.1	101.8	**Average:** 101.6
0.1	100.0		0.1	101.7	
0.1	100.0		0.1	101.5	
0.1	100.30	**RSD percent:** 0.10	0.1	101.4	**RSD percent:** 0.10
0.1	100.10		0.1	101.7	
0.1	100.0		0.1	101.5	

RSD – relative standard deviation

Intermediate Precision/Ruggedness

Six injection formulation sample solutions were prepared at concentration level of 100% (0.1 mg/ml). For every sample, the assay of levetiracetam according to the developed method was assessed by two different chemists in two different laboratories. The percent RSD

for assay results were assessed. The findings are shown in Table 5. The results confirmed that the procedure met the criteria (percent RSD was <2.0%) for intermediate precision/ruggedness.

Table 5: Levetiracetam intermediate precision/ruggedness details

Laboratory	Injection formulation with label claim 5 mg/ml			Injection formulation with label claim 15 mg/ml		
	Concentration (mg/ml)	**Assay (%)**	**Statistical assessment**	**Concentration (mg/ml)**	**Assay (%)**	**Statistical assessment**
Analytical research and development laboratory	0.1	100.0		0.1	101.8	
	0.1	100.0		0.1	101.7	
	0.1	100.0	**Average:**	0.1	101.5	**Average:**
	0.1	100.3	100.3	0.1	101.4	100.9
	0.1	100.1		0.1	101.7	
	0.1	100.0		0.1	101.5	
Quality control laboratory	0.1	100.4		0.1	100.0	
	0.1	100.6		0.1	100.3	
	0.1	100.6	**RSD percent:**	0.1	100.0	**RSD percent:**
	0.1	100.5	0.30	0.1	100.4	0.8
	0.1	100.6		0.1	99.9	
	0.1	100.8		0.1	100.2	

RSD – relative standard deviation

Accuracy

Samples for accuracy study were made by adding levetiracetam reference drug to excipient solution at concentrations of 50% (0.0507 mg/ml), 100 % (0.1014 mg/ml) and 150% (0.1521 mg/ml) relating to test concentration (0.1mg/ml of levetiracetam). For every sample, the assay of levetiracetam according to the developed method was assessed. For individual preparations, the percent recovery was measured at every concentration level and an average of the percent recovery was estimated. For every concentration level the percent RSD for percent recovery was also estimated. The findings are shown in Table 6. The results confirmed that the procedure met the criteria (percent recovery was 97.0 – 103.0% and percent RSD was <2.0%) for accuracy.

Table 6: Levetiracetam accuracy and recovery details

Level (with relating to test concentration – 0.1 mg/ml)	Added amount (mg/ml)	Found amount (mg/ml)	Recovered percent (%)	Statistical assessment
50	0.507	0.0519	102.4	**Mean recovery:**
		0.0519	102.4	102.4
		0.0519	102.4	
		0.0521	102.8	**RSD percent:**
		0.0519	102.4	0.20
		0.0519	102.4	
100	0.1014	0.1024	101.0	**Mean recovery:**
		0.1023	100.9	100.9
		0.1023	100.9	
		0.1023	100.8	**RSD percent:**
		0.1023	100.9	0.10
		0.1026	101.2	
150	0.1521	0.1554	102.1	**Mean recovery:**
		0.1553	102.1	100.9
		0.1525	100.3	
		0.1524	100.2	**RSD percent:**
		0.1525	100.3	0.90
		0.1528	100.4	

Specificity

To prove that the excipients and diluent do not interfere with the assessment of levetiracetam, the pure substance (levetiracetam - 0.1 mg/ml), diluent, injection formulation (levetiracetam - 0.1 mg/ml) and excipient solution was analyzed individually by the suggested method. The levetiracetam (0.1 mg/ml) spiked in the excipient solution was also analyzed by the suggested method. The retention times in all the cases were compared to establish specificity [Figures 1a-1e].The results confirmed that the procedure met the criteria for specificity because no peaks due to the excipients/diluent were noted to be interfering with the assessment of levetiracetam.

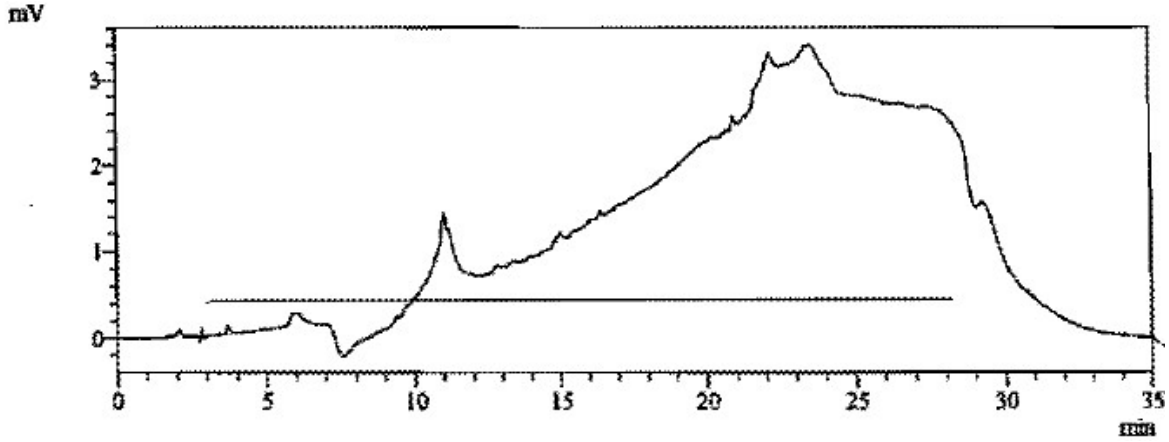

Figure 1a: Typical diluent chromatogram

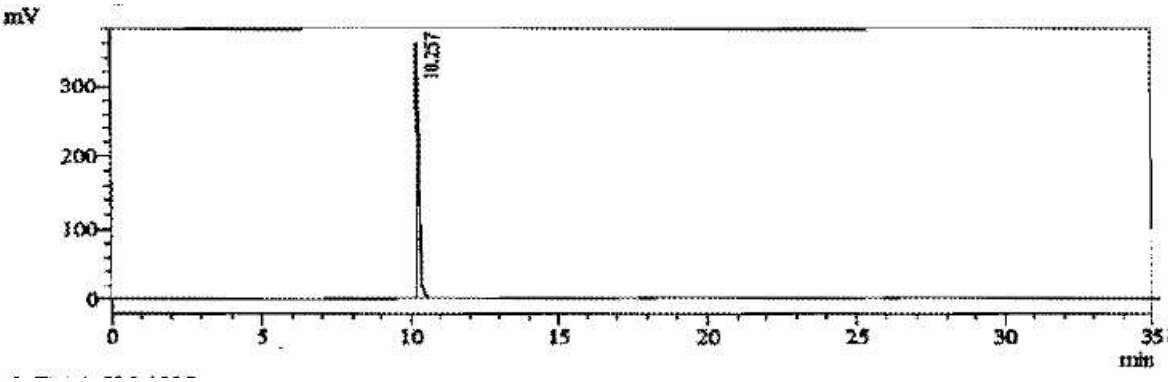

Figure 1b: Typical standard (levetiracetam - 0.1 mg/ml) chromatogram

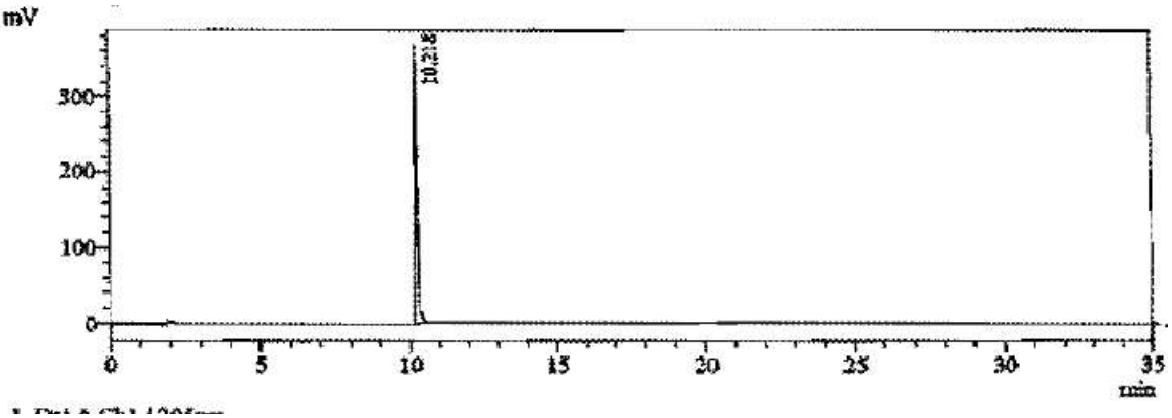

Figure 1c: Typical injection formulation (levetiracetam - 0.1 mg/ml) chromatogram

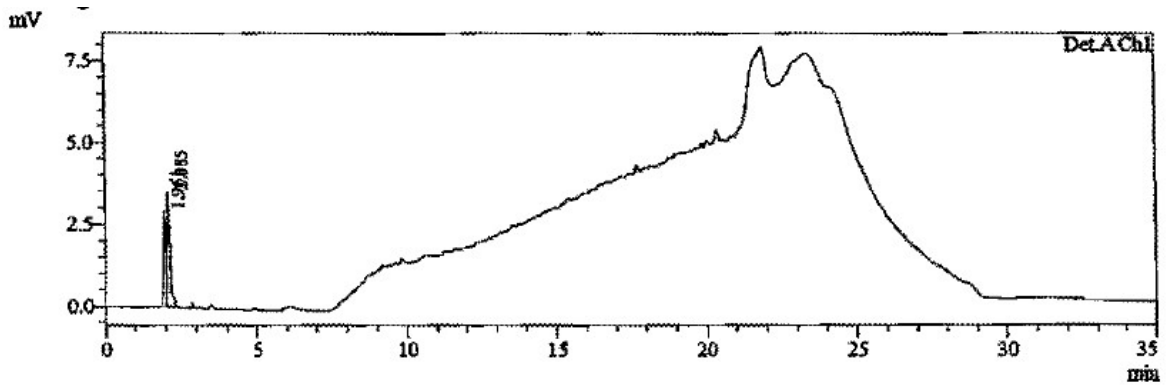

Figure 1d: Typical excipient solution chromatogram

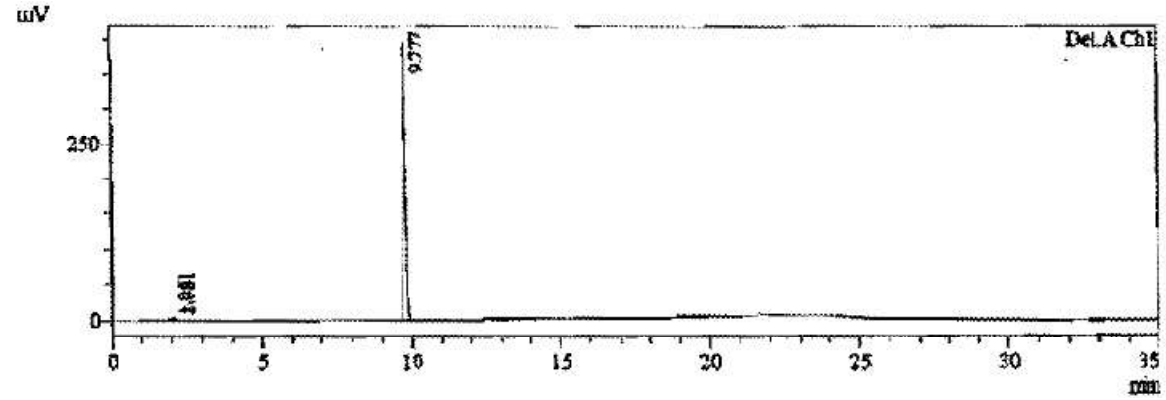

Figure 1e: Typical excipient and levetiracetam (0.1 mg/ml) solution chromatogram

StabilityIndicating Characteristic Feature

Developed method's stability indicating characteristics was demonstrated by its ability to resolve levetiracetam from its degradation products. For this, control (undegraded injection sample solution), dry heat and light exposed injection formulation samples prepared were assessed as per the method developed. Excipient placebo solutions spiked with levetiracetam were exposed to acid, base and peroxide stress conditions were also assessed as per the method developed. The chromatograms of all the degradation studies were shown in Figure 2a – 2f. The percent assay, percent degradation, peak purity and spectral match were determined in all conditions of stress [Table 7]. The peak purity index and similarity index obtained for the degraded stress samples was >0.990 indicating pure peaks devoid of any co-elution and spectrally matched peaks, respectively.The results confirmed that the procedure met the criteria for stability indicating feature because no peaks due to the levetiracetam degradation products were co-eluting with peak of levetiracetam.

Table 7: Method's stability indicating feature and stability of levetiracetam details

Sample	Assay (%)	Degradation (%)	Peak purity index	Similarity index
Control	99.9	-	1.000000	0.999998
UV exposed	101.8	-1.8	1.000000	0.999999
Dry heat exposed	98.6	1.4	0.999999	0.999997
Acid exposed	95.0	5.0	0.999999	0.999992
Base exposed	93.3	6.7	1.000000	0.999995
Peroxide exposed	96.8	3.2	0.999999	0.999994

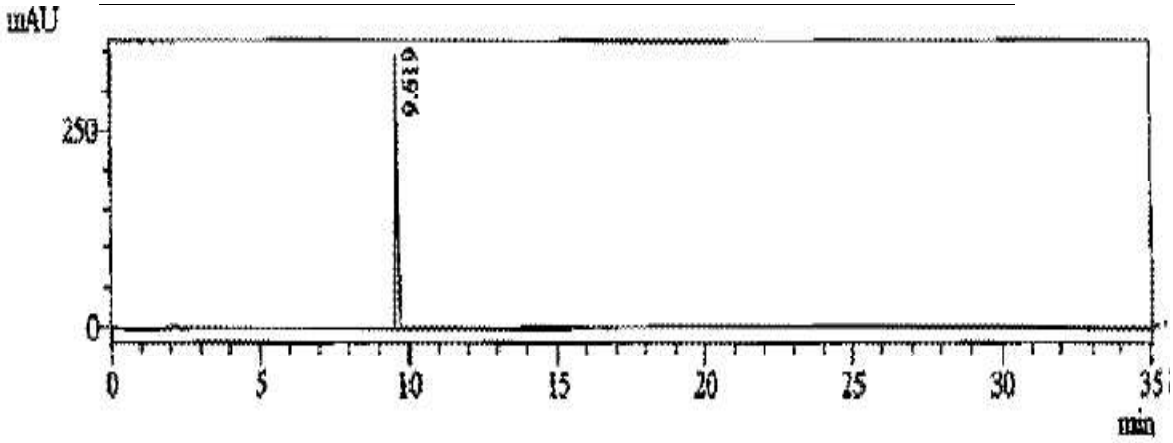

Figure 2a: Typical control (undegraded) chromatogram

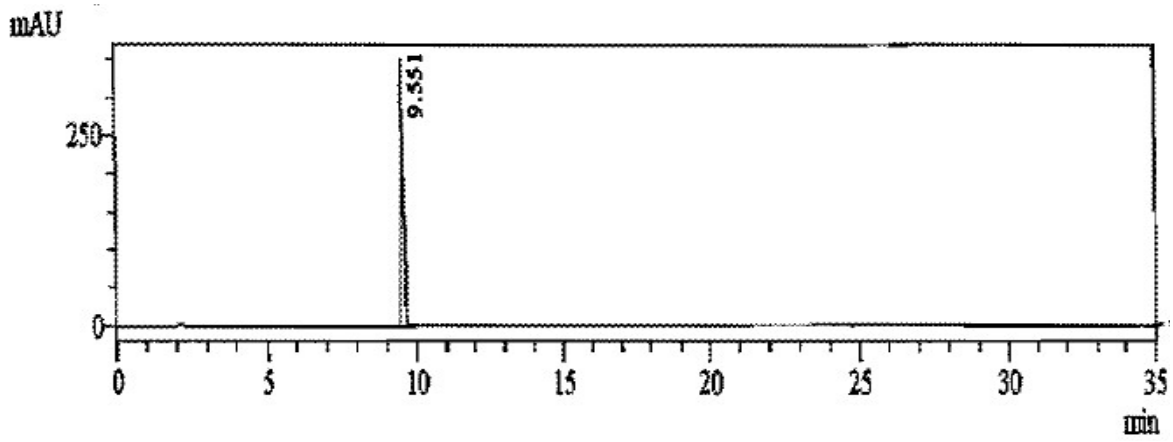

Figure 2b: Typical light exposed sample chromatogram

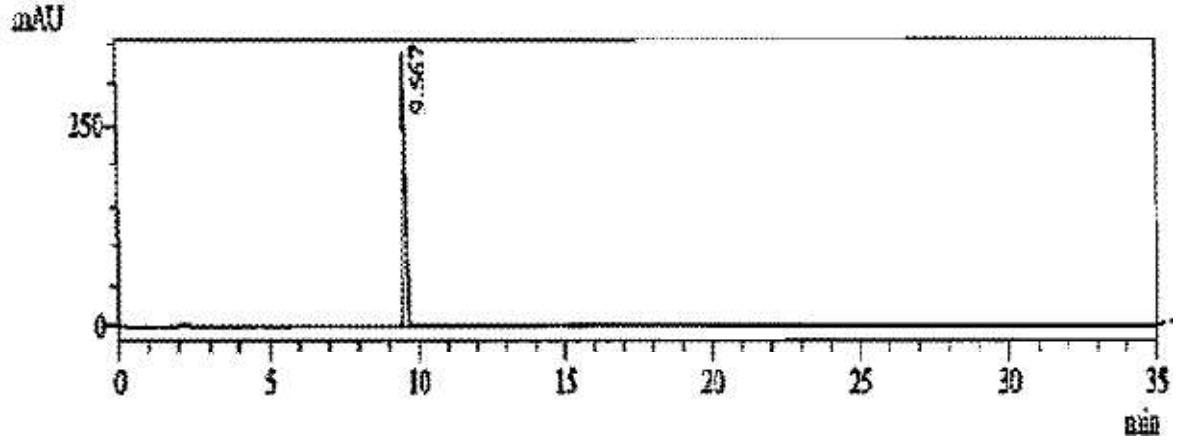

Figure 2c: Typical dry heat exposed sample chromatogram

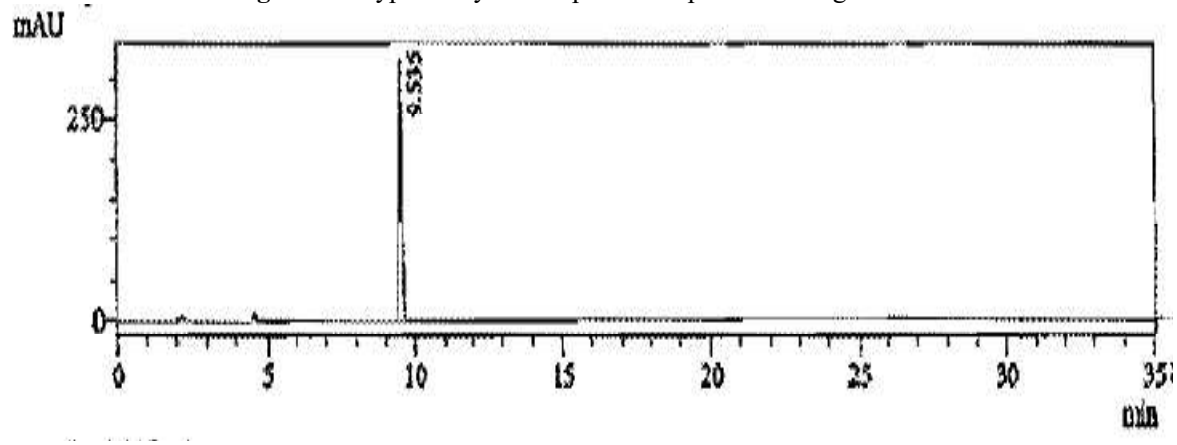

Figure 2d: Typical acid exposed sample chromatogram

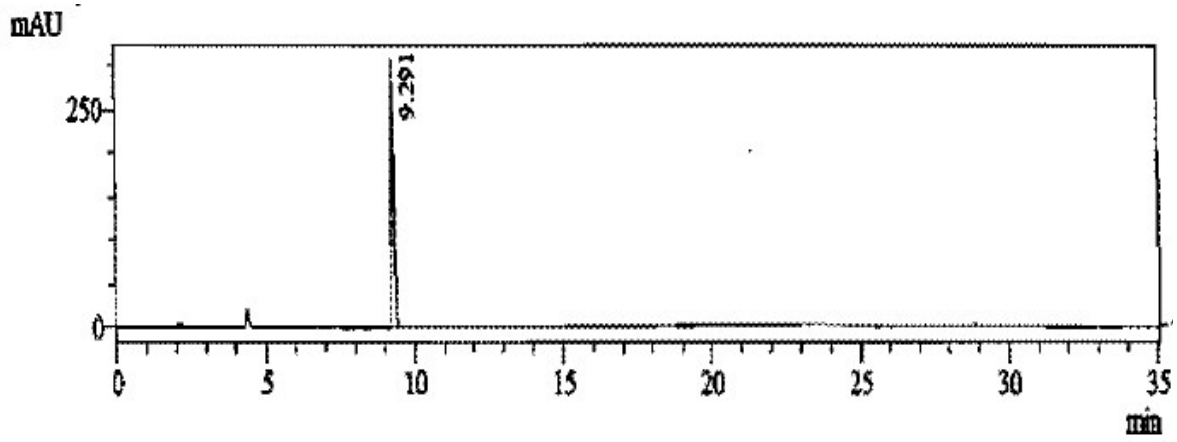

Figure 2e: Typical base exposed sample chromatogram

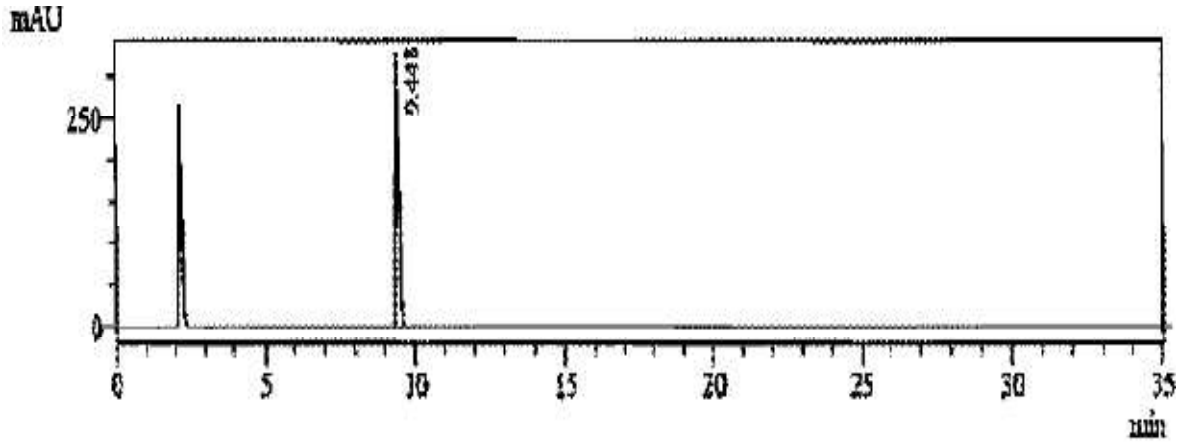

Figure 2f: Typical base exposed sample chromatogram

Robustness

The effects of minor tolerable change in column oven temperature, column lot, buffer pH and flow rate on levetiracetam assay by proposed method were evaluated by assessing the standard levetiracetam solution (0.1 mg/ml). The difference between the assay results of levetiracetam in minor altered conditions with optimized conditions [Table 8] was established. The results confirmed that the procedure met the criteria (percent difference was <2.0%) for robustness.

Table 8: Levetiracetam robustness details

Condition applied	Assay (%)	Difference (%)
Variation in column lot		
YMC PACK AQ Column ID: LCF 103/12	100.0	0.4
YMC PACK AQ Column ID: LCF 104/12	100.4	
Variation in column oven temperature		
20 °C (optimized)	102.0	1.3
25 °C	100.7	
Variation in flow rate		
0.8 ml/min	100.7	1.3
0.9 ml/min (optimized)	102.0	-
1.0 ml/min	100.6	1.4
Variation in mobile phase buffer pH		
pH 5.3	102.0	0.0
pH 5.5 (optimized)	102.0	-
pH 5.7	101.7	0.3

CONCLUSION

The new stability indicating RP-HPLC method developed forthe assay of levetiracetam in injection formulation was found to be accurate and precise. The procedure was noticed to be linear for levetiracetam assay over the range of 0.0519 mg/ml to 0.1557 mg/ml.The procedure is repeatable, rugged, specific and stability indicating for levetiracetam. The method is also robust for variations in column lot, flow rate, column oventemperatureand buffer pH.

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